590

MAPLE SIRUP. III. PRELIMINARY STUDY OF THE NONVOLATILE ACID FRACTION[a]

W. L. PORTER, M. L. BUCH, AND C. O. WILLITS
Eastern Regional Research Laboratory,[b]
Philadelphia, Pennsylvania

Relatively little scientific work has been done to improve the quality and uniformity of maple sirup or to develop improved processing methods. Much of the literature *(2, 5, 12)* relates to control measures, such as prevention of adulteration and detection of contamination. Since the value of maple sirup lies in its distinctive flavor, a better knowledge of the flavoring substances is necessary for improvement of processing practices. Maple sirup consists mainly of sucrose and water; the flavoring material is present among the trace constituents. Some investigations on maple flavor have been reported *(2, 3, 5)*, but the results were not complete. Findlay and Snell *(3)* reported that the flavor is developed during heat evaporation of sap to sirup. Our preliminary work demonstrated that sap as it leaves the maple tree has no maple flavor. An essentially flavorless sirup was produced by vacuum evaporation at room temperature, confirming the observation of Findlay and Snell. We further confirmed it by production of a completely flavorless sirup by freeze drying. When heated at atmospheric pressure, this sirup acquired typical maple flavor, proving that during the production of sirup the flavor is developed by heating the maple sap at atmospheric pressure.

These facts were the basis of our hypothesis that maple flavor is the result of a browning (Maillard) reaction. Production of a synthetic sirup having a maple-like flavor by Barnes and Kaufman *(1)* lends support to this assumption. Production of high-flavored maple sirups by heat treatment of commercial maple sirups *(11)* may be further evidence of the role of the browning reaction.

To investigate the possibilities of the browning reaction between amino acids, sugars, organic acids, and aldehydes, it was necessary to study in detail each of these groups as it occurs in maple sap. The progress report given here on the nonvolatile acid fraction is the first of a series of papers dealing with the composition of maple sap and maple sirup and is the third of a general series of papers on maple sirup issued from this laboratory *(11)*.

The literature on the nonvolatile organic acids of maple sirup indicates that there is considerable uncertainty as to both the total number and the identity of these acids *(7, 9)*. Most of the acids reported were identified by the time-honored method of (a) extraction with ether after addition of acid equivalent to the total alkalinity of the ash, (b) distilla-

---

[a] Report of a study made under the Research and Marketing Act of 1946.

[b] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

tion of the volatile acids and precipitation of the nonvolatile acids as the lead salts, and (c) acid recovery by esterification and fractionation of the esters *(7)*. These procedures, even for large volumes of starting material (35 to 40 liters), may not be of sufficient sensitivity and accuracy to detect the acids existing in trace amounts. Methods employed in this work, in which only a fraction of the above amount of sirup was used were (a) ion-exchange techniques to separate the organic acids in a relatively pure form, and (b) paper chromatography for identification of the acids.

EXPERIMENTAL

Ion Exchange Methods. Sixty ml. of cation resin, Dowex 50,[c] in the spherical form was employed in the (H^+) ion cycle in a 17 x 0.75-inch ion-exchange tube equipped for downflow exhaustion and regeneration and for backwashing at 50% bed expansion. A flow rate of 0.26 ml. per minute per ml. of resin was used throughout the experiments. For regeneration, four bed volumes of 2*N* HCl were used, and the columns were washed with distilled water until the eluate was free of chloride ion.

Sixty ml. of anion resin, Duolite A-4, was used for the acid exchange. The bed volume, column dimensions, and rate of flow were the same as for the cation column. For regeneration, four bed volumes of 1*N* NaOH were used, and the columns were washed with distilled water until the eluate was acid to phenolphthalein.

Paper Chromatographic Methods. The method of Lugg and Overell *(6)* was followed with some variations. Strips of Whatman No. 1 paper (6 x 22.5 inches) were used as the solvent support. Downflow operation, with 3 hours of equilibration of the papers with the aqueous phase, was employed in all cases. After the papers were dried in a hood for 2 hours at room temperature, the spot positions of most of the acids were identified by spraying with bromophenol blue (0.04%) adjusted to slight alkalinity. Naphthoresorcinol (0.1%) in 1*N* alcoholic H_3PO_4 was used to indicate the presence of keto and uronic acids. Ammonium vanadate (2%) in water indicated the presence or absence of tartaric acid by the characteristic orange spot. Ammoniacal silver nitrate sprays produced characteristic colors with some acids after heating at 105°C. for 10 minutes, and additional information was obtained by viewing the papers, processed with this reagent, under ultraviolet light. Ninhydrin (0.1%) in n-pentanol was used for amino acids. Separate chromatograms were made for each detecting (spray) material.

Procedure. Two hundred g. of a commercial maple sirup was diluted with water to 15% solids content and run into the cation column at the stated flow rate, and the effluent was passed through the anion column immediately. The two columns were washed in series with distilled water until the effluent was free of acids and of sugars. The cation column was eluated with 2*N* HCl and washed with water. After concentration in vacuo, this eluate contained the inorganic cations and the neutral and basic amino acids. The organic acids retained by the anion column were eluted with 1*N* NaOH, and the column was washed with water until the eluate was acid to phenolphthalein. The eluate and washings containing the sodium salts of the organic acids were passed through a regenerated cation (H^+) column to form the free acids. These were washed from the column with distilled water, washing being continued until the eluate was free of acid.

The acid fraction was concentrated by boiling until test papergrams indicated the acid concentration that would give the best resolution. This also removed the volatile acids; any insoluble material formed was removed by filtration. Then 0.01 ml. of the acid concentrate was placed on the paper by means of a Breed and Brew pipette, and after the solvent had evaporated, the paper was hung from the solvent tray in a chromatographic tank containing only the aqueous phase of the solvent system obtained from the mutual saturation of equal volumes of n-pentanol and 5*M* formic acid. After the papers were equilibrated with the vapor of the water phase, the chromatograms were developed for 18 hours (the organic phase of the solvent system was used), removed from the tank, dried, and sprayed with the appropriate detecting solution. R_F values

[c] Mention of commercial products does not imply that they are indorsed or recommended by the Department of Agriculture over others of similar nature not mentioned.

were calculated for each spot. Final identification was made by comparison of each spot with that obtained from a known compound.

Table 1 shows the number of nonvolatile acids in the anion fraction separated by the papergrams, the R_F value of each spot, the R_F values of the reference compounds, the method of indicating spot positions, and whether reported previously in the literature. These results were obtained using a commercial No. 2 grade maple sirup.

TABLE 1

Nonvolatile organic acids in maple sirup

Spot No.	R_F	Acid	R_F value of reference compound	Indicator[d]	Presence reported in literature
1	0.03	Unidentified	.....	1, 2	.....
2	0.07	Unidentified	.....	3	.....
3	0.11	Unidentified	.....	1, 2	.....
4	0.16	Unidentified	.....	1, 4	.....
5	0.26	Citric	0.24	1, 5	Nelson(7)
6	0.36	Malic	0.35	1, 5	Nelson(7)
7	0.44	Glycolic or 1:2 dihydroxybutyric[e]	0.45	1, 5	No
8	0.47	Unidentified	.....	1	.....
9	0.50	Unidentified	.....	1	.....
10	0.64	Succinic	0.63	1, 5	Nelson(7)
11	0.68	Unidentified	.....	1	.....
12	0.82	Fumaric	0.82	1, 5	Nelson(7)

[d] Indicators: 1, bromophenol blue; 2, naphthoresorcinol in alc. H_3PO_4; 3, ninhydrin; 4, ammonium vanadate; 5, ammoniacal silver nitrate.

[e] Subject to final proof of identity by isolation and analysis.

DISCUSSION

Four of the twelve acids shown to be present in commercial maple sirup have been identified by their R_F values as citric, malic, succinic, and fumaric acids. The acid in spot 1 is a uronic acid, as indicated by the slow rate of movement and by the color produced with naphthoresorcinol. The acid in spot 7 could be glycolic acid or 1:2 dihydroxybutyric acid. Isolation of this compound is necessary for final confirmation. The acid in spot 2 is probably an amino acid, as indicated by the ninhydrin reaction, and is probably dicarboxylic, since it is not held by the cation ion exchanger under these experimental conditions. It has a low R_F value. This compound, however, may be a glycoside of the type suggested by Gottschalk and Partridge *(4)* formed during browning. Nelson reported the presence of tartaric acid, which should chromatograph with an R_F of 0.16. A spot with such an R_F was found but not identified as tartaric acid, since a negative result was obtained with the ammonium vanadate spray. Tricarballylic acid has been reported in the calcareous precipitate formed during maple sirup production *(8)* but not in maple sirup *(7)*, and no trace of it was found in our work. Qualitative studies with Fancy, No. 1, and No. 3 sirups indicate the same acids to be presnt, although probably in different proportions. Work is being continued on the isolation and identification of the remaining acids by chromatography, X-ray diffraction, ultimate analyses, and spectrophotometric examination.

SUMMARY

In a study of the browning reaction in the formation of maple flavor, the composition of the nonvolatile organic acid fraction of maple sirup was investigated by means of ion-exchange and paper chromatography. The presence of citric, malic, succinic, and fumaric acids has been confirmed. Glycolic acid or 1:2 dihydroxybutyric acid is possibly present. Further work on the isolation and identification of this compound is in progress. The absence of tartaric acid and tricarballylic acid has been suggested. The techniques employed are sensitive to the acids present in trace amounts and there has been no experimental evidence to indicate alteration of the naturally occurring acids.

LITERATURE CITED

1. BARNES, H. M., AND KAUFMAN, C. W. Industrial aspects of browning reaction. *Ind. Eng. Chem.*, **39**, 1167 (1949).
2. BOIS, ELPHEGE, AND DUGAL, LOUIS-CHARLES. Steam distillation of maple sirup. *Naturaliste can.* **68**, 209 (1941).
3. FINDLAY, G. H., AND SNELL, J. F. Some constituents of the sap of the sugar maple (A. saccharum). *Can. J. Res.*, **30**, 13B, 269 (1935).
4. GOTTSCHALK, A., AND PARTRIDGE, S. M. Interaction between simple sugars and amino acids. *Nature*, **165**, 684 (1950).
5. HORTUET, J. The chemical composition of maple sirup and maple sugar, methods and analysis for detection of adulteration. *J. Am. Chem. Soc.*, **26**, 1523 (1904).
6. LUGG, J. W. H., AND OVERELL, B. T. "One" and "two-dimensional" partition chromatographic separations of organic acids on inert sheet support. *Australian J. Sci. Research*, Series A, Phys. Sciences, **1**, 98 (1948).
7. NELSON, E. K. Acids of maple sirup. *J. Am. Chem. Soc.*, **50**, 2006 (1928).
8. NELSON, E. K. Acids of maple sugar sand. *J. Am. Chem. Soc.*, **50**, 2028 (1928).
9. VON LIPPMAN, E. O. Organic acids from the sap of the sugar maple. Ber. **47**, 3094 (1914).
10. WILLITS, C. O., AND PORTER, W. L. Maple sirup. I. Research program on maple products at the Eastern Regional Research Laboratory. United States Department of Agriculture, Bur. Agr. and Ind. Chem., AIC-268 (Eastern Regional Research Laboratory). (Processed.) (1950).
11. WILLITS, C. O., AND PORTER, W. L. Maple sirup. II. A new high-flavored maple sirup. United States Department of Agriculture, Bur. Agr. and Ind. Chem., AIC-269 (Eastern Regional Research Laboratory). (Processed.) (1950).
12. WILLITS, C. O., AND TRESSLER, C. J., JR. Sources of lead in maple sirup and a method for its removal. *Food Research* **3**, 449 (1938).